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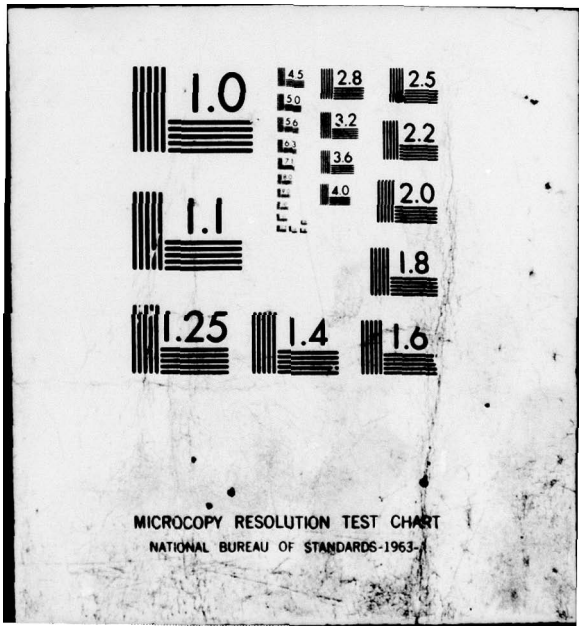
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THE ORIGIN OF CATARACTS IN THE LENS FROM INFRARED LASER RADIATION

Progress Report

M. L. Wolbarsht
M. A. Orr
I. B. C. Matheson

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D. C. 20314

Contract DAMD 17-74-C-4133

Duke University Medical Center
Durham, North Carolina 27710

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Progress Report
Contract #DAMD 17-74-C-4133
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SUMMARY

The earliest possible changes detectable in cataract formation are a depletion of the soluble α crystallin lens proteins by gel electrophoresis analysis. Rabbit lenses exposed in vivo to different power levels from CW neodymium laser (1060 nm) at various power levels were used to produce the changes in vivo and in vitro.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences--National Research Council.

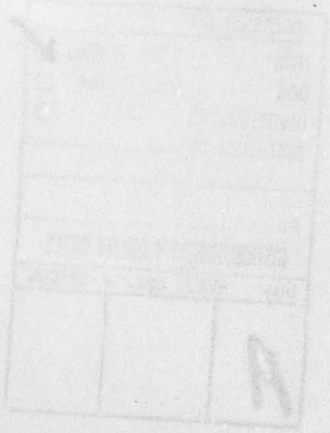

TABLE OF CONTENTS

INTRODUCTION

The purpose of this work was to establish the lenticular protein changes which occur as a precursor to cataracts formed following exposure to IR laser radiation. These initial changes will be used as a standard to judge the harzardous levels of exposure to a CW neodymium (1060 nm) laser source.

No widely accepted mechanism of the formation of cataracts following infrared irradiation now exists. Earlier workers concluded that both light and infrared radiation are absorbed in the pigment epithelium of the iris and converted to heat, damaging the adjacent epithelium of the lens and later causing a cataract to develop (Langley et al., 1960). Others have maintained that the heat cataract is due to the direct absorption of radiant energy by the lens (Vogt, 1930, Wolbarsht et al., 1977).

The cataract induced by infrared irradiation starts with cobweb-like strands in the posterior cortex and ultimately forms a complete opacity resembling a senile cataract (Hanna, 1975). Like the senile cataract there is a decrease in the relative amount of soluble protein.

In an occupational setting, acute exposures would not occur. It has been calculated that the rise of temperature in the iris and anterior portions of the lens from the energy levels of chronic occupation exposures is very slight, less than 1°C. In any case, the thermal theory of cataractogenesis assumes that this increase in temperature of the anterior portion of the lens (which is in contact with the heated iris) will in time produce denaturation of a sufficient amount of lens protein to initiate a cataract. The cataract induced by infrared irradiation is throught to be similar to senile cataract in that there is a loss of ascorbic acid and a decrease in the relative amount of soluble protein.

In contrast with this theory involving heat conduction of the iris, a possibility also exists that the infrared radiation is absorbed directly by the lens. Absorption by the lens is low in the near infrared, but at 1300 nm it abruptly becomes high. At 1060 nm the absorption is approximately 20%. Since the increase in temperature in the lens during chronic exposures is so minute, protein denaturation or inactivation of enzyme systems due to thermal stress appears unlikely. It is rather more likely that some specific chemical process is stimulated or suppressed by the absorption of the infrared radiation. Furthermore, it is possible that the photochemical stress of the infrared irradiation could cause local protein damage resulting in a configurational change, or the production of abnormal (miscoded) proteins. The soluble lens proteins, which include the α, β, and γ crystallins, comprise over 90% of the dry weight of the lens. The function of the lens crystallins is to produce a matrix which is fully transparent to visible light, and remains so during lens accommodation. It seems likely that precisely ordered optical interactions among the various lens proteins would be required to produce the

constructive interference necessary to produce such a transparent system. Any disruption in this structure sufficient to cause destructive interference could lead to a measurable loss of transparency, especially since the repair processes go on at a low rate.

The possibility of a non-thermal process in initiating IR cataract formation suggests a similarity between IR cataracts and ultraviolet-induced senile or brunescent cataracts. Brunescent cataracts have been widely studied and a review of these studies has suggested programs to be used in the study on infrared laser cataractogenesis. Briefly the changes noted in brunescent (senile) cataracts are a decrease in the total lens proteins mostly in the high molecular weight moiety (Dawson, 1962), accompanied by a loss of ascorbic acid and a buildup of glutathione and the insoluble albuminoid protein fraction (Dische et al., 1956). In a wide variety of cataracts (brunescent, X-ray, UV, glass-blowers', galactosemia-induced) the α-crystallin converts to the insoluble albuminoid form. This conversion probably involves a conformational change in the portion of the protein that masks the sulfhydral group (Merola and Kinoshita, 1957) and SH, once exposed, helps form a complex between the soluble α-crystallin and the trace amounts of glycoprotein present (Dische, 1965).

The human lens is not normally subjected to radiation longer than 2000 nm or shorter than 293 nm, as the cornea absorbs very strongly in these regions. However, the lens absorbs most of the radiation from 300 to 400 nm and between 1100 and 1400 nm. The relative effectiveness for catatactogenesis of various portions of the near infrared as a function of wave length is not known at present.

It seems likely that within the UV sensitive range the relative quantum efficiency for cataractogensis has a relation to the photon energy, with the higher energy photons being the more effective. Most important, however, may be any strong absorption bands in tryptophan. In brunescent cataracts, the photooxidation of tryptophan proceeds through several degradation steps: kynurenine to 3-hydroxykynurenine, and its subsequent degradation via further hydrolyzed products of kynurenine. These latter degradation products can be detected by ultraviolet fluorescence techniques (Kurzel et al., 1973, Kurzel et al., 1972). Glycoproteins have also been implicated with senile cataract formation (Carlin and Cotlier, 1971) and particularly with the glycosidase activity in lenses. This enzyme activity may be specifically related to the excited state of the glycoside of 3-hydroxykynurenine. Glycoproteins may also act as the source for "active" glucose molecules originally suggested by Spiro and Spiro (1968) and found by Spector et al. (1971) as the trigger for the onset of α-crystallin aggregate formation. However, the "active" glucose binds the low molecular weight crystallin components to each other to form the larger albuminoid moieties.

In the aging human lens, the decreasing amount of α-crystallin and increasing amount of non-soluble albuminoid suggests that α-crystallin

is being converted into albuminoid. The amino acid composition of α-crystallin and albuminoid of the human lens indicate that they compare closely, though there is but sufficient variation to suggest that albuminoid is not entirely composed of α-crystallin, but probably contains some β- and γ-crystallin as well (Clark et al., 1969). A high-molecular weight (HM) protein fraction composed of α-crystallin polypeptides has been found in calf lens. Furthermore, a urea-soluble, reduced protein was found in the rabbit lens which reveals a complex pattern with polyepetides in the α-crystallin as well as in the β-crystallin region. A study by Liem-The and Hoenders (1974) shows the similarity in subunit composition of α-, HM-crystallin and the urea-soluble protein. As suggested earlier, a transition may take place from α-crystallin to HM-crystallin, with HM-crystallin serving as an intermediate in the process of insolubilization of the crystallins. Extensive reaggregation or alteration in the chemistry of the lens would lead to insolubilization and opacification.

The major work during this period was the study of the effect of IR laser irradiation on lens proteins with longer exposure times and lower energy levels than previously used. Similarities were noted in the protein changes produced by the laser to those produced in concurrent studies of incubation of lenses at elevated temperatures and to the incubated lenses exposed to a broad band IR light source.

PREVIOUS RESULTS

The initial portion of this program involved amino acid analysis of cataracts produced by a CW neodymium laser (1060 nm). The results showed no marked changes in the amino acid distributions as compared with a normal lens. This suggests that the chief mechanism of cataract formation is most likely protein denaturation. Further laser studies produced cataracts which were analyzed by isoelectric focused thin layer gel electrophoresis. Analysis of the cataractous portions of the lens showed an almost complete disappearance of the soluble crystallins (α, β_H, β_L, and γ). This indicates a strong linkage between opacity formation and the complete precipitation of these crystallins. In the clear portions of the cataractous lenses the concentrations of the soluble proteins were almost unchanged except for the β_H crystallin fraction which could be the earliest indication of cataractogenesis.

Concurrent experiments on the possible direct effect of heat on the lens have been conducted by incubating extracted and homogenized lenses at temperatures from 37° to 45°C. The higher temperatures showed marked diminution of the α crystallin fraction (Wolbarsht, 1978).

Results of column chromatography run on normal and cataractous lenses indicate that the disappearance of the soluble crystallins is probably by aggregation into insoluble albuminoid forms with a molecular weight greater than 1,000,000.

We have also shown that whole rat lenses maintained in organ culture for three days at a controlled temperature of 40°C showed a loss of a possible β crystallin fraction and a decrease in the α crystallin motility. Rat lenses maintained in organ culture for seven days at 38.5°C showed no change from the control. These results determined by isoelectric focused thin-layer gel electrophoresis indicates that the threshold temperature for inducing changes in the rat lens protein is between 38.6°C and 40.0°C. The disappearance of the β band due to the heat stress also fits in with a hypothesis (Liem-The and Hoenders, 1974) of how the transition of the soluble crystallin fraction into the insoluble albuminoid protein fraction in the rabbit lens occurs. The rat lenses were used as a preliminary test to determine the temperture which will initiate cataractogenesis.

Concurrent with these experiments, exposures from a CW neodymium laser (1060 nm) were used to determine what energy levels were necessary to produce protein changes in the rabbit lens. (The energy level in joules of each exposure was determined by multiplying the power level times the exposure time.) At a power level of 2.00 W, an exposure of 50 sec. (100 joules) produced an opacity over the entire anterior surface of the lens. A lower power level (1.75W) and an increased exposure (90 sec.) (157.5 joules) produced the same result whereas a power level of 1.75 W at 50 sec. (87.5 joules) produced only a peripheral opacity. Analysis of these lenses using sodium dodecyl sulfate (SDS) gel electrophoresis, and isoelectric focused thin layer electrophoresis with and without urea showed protein changes in the α crystallins occurring in lenses exposed to energy levels of 100 joules or more.

PRESENT PERIOD:

EXPERIMENTAL METHODS

Analysis of Lens by Gel Electrophoresis.

Our current research program has sought to identify a similar chain of protein degradation products connected with infrared cataractogenesis. Analysis of the lens protein has been accomplished in our previous studies primarily through gel electrophoresis. The electrophoretic process, by separating the protein fractions into identifiable bands, allows us to determine which protein constituents have been affected, which have decreased in concentration, and what new fractions are appearing. Some of the changes which occur in the various crystallins can be conveniently studied after they are separated by isoelectric focused thin gel electrophoresis of the homogenized lens. This technique is now widely used to separate and characterize such protein mixtures. It is basically electrophoresis in a pH gradient, which separates the various proteins according to their isoelectric points. As the thin layer polyacrylamide gel can be efficiently cooled even with high current densities, the experimental time can be markedly shortened. The exact procedure used was established by Zigler (1975).

Gel electrophoresis of proteins denatured to their constituent polypeptides by sodium dodecyl sulfate (SDS) is another widely used procedure. The electrophoretic mobility of such denatured proteins is linearly related to their molecular weight which makes identification of the resultant bands much easier.

Details of In Vivo Laser Exposures

All laser exposures were made with a CW neodymium-YAG laser (Holobeam Model 250). It is a multi-mode CW laser with the majority of its output in a 1065 nm beam, approximately 3 mm in diameter at the exit port. The beam was enlarged in collimated form to 24 mm by means of an 8x beam expander (Edmund Scientific). In a previous report (Wolbarsht et al., 1977) the laser output is shown as a function of the lamp input power to indicate the reproducibility of the output as a function of the input power. The power input was measured by a Scientech (Boulder, Colorado, Model 360) disc calorimeter which sampled the back beam of the laser. The ratio of the front to back beam of the laser output was measured periodically so that the front beam output could be accurately calculated by measuring the back beam power with an appropriate correction factor. The disc calorimeter was calibrated absolutely by passing a known current through a built-in heating resistor noting the calorimeter output. The measurements of laser power were relatively unaffected by the back reflection from the beam expander which had a constant 10% total air-glass reflection from all the surfaces.

The relative position of the eye of the exposed rabbit to the laser is the same as used previously (Wolbarsht et al., 1977). The rabbits weighing 2-3 lbs. were pigmented, from a mixed litter, and 8 weeks old. They were anesthetized with 0.6 cc. sodium pentobarbitol before exposure. The eyeball and cornea were numbed by Alacaine, and Duke Mix dilator (phenylepheine, mydriasil; 1:1) W as used. Even with the application of Duke Mix, the iris constricted to some degree with the laser irradiation.

Following irradiation, the animal was sacrificed, the eyes removed and the lens extracted. Each lens was homogenized in 3.5 ml. H_2O, centrifuged at 9,000 rpm, and the supernatant containing the water soluble lens protein saved for analysis by gel electrophoresis. The details of the analysis are given in a previous report (Wolbarsht et al., 1977).

EXPERIMENTAL RESULTS FROM PRESENT PERIOD: DETAILS AND DISCUSSION

Exposures at a power level of 0.233 W very quickly produced obvious thermal damage, demonstrating that use of such high levels would cause so much damage as to swamp out. At 0.233 W, exposure for two min. (28 J) produces an opacity on the edge of the cornea and indications of a lenticular opacity beginning underneath the corneal opacity. Exposure for five minutes at this same power level evidently

heated the eye speculum enough to burn the eye lids. In both cases, the SDS gel analysis produced no conclusive results, as all bands were affected.

At 0.16 W, an hour of laser exposure produced an almost complete lenticular surface opacity both anterior and posterior. Only a central disc on the posterior side remained clear. This time power combination represents an exposure of 576 joules. At this level, a great deal of the soluble protein is converted into insoluble protein and does not produce band patterns on the SDS gel. Such an energy level is too destructive to the lens proteins to enable identification of the subtle changes sought.

Laser exposure at 0.1 W for 17.5 minutes produced a ring-shaped opacity on one edge of the cornea which corresponded to a lenticular surface opacity directly underneath. This time/power combination represents an exposure level of 105 joules (0.1 W X 1050 sec.). The SDS gel electrophoresis shows band 7 (presumably an α crystallin) decreasing in concentration at this energy level. This observation is identical to results at the same energy levels reported previously with higher wattage, but shorter (and fractionated) exposure times (2 W X 50 sec). This indicates that the dose is the important parameter, and that reciprocity between power and time holds over more than 1 order of magnitude.

Additional exposures (from 15 to 23 min. duration) all showed that at about 100 joules there was a marked decrease in the α crystallin protein (band 7 on the electrophoresis gel). This obviously dose related type of relationship argues very heavily against any type of thermal model for infrared cataractogenesis. The exact mechanism by which the α crystallin is affected is at the present unknown. However, exposures for longer periods of time and lower levels may fractionate the lens response. The change may be confined to the α crystallin, or alternatively, studies on α crystallin alone may show how the infrared affects this molecule. However, it should be kept in mind that extracted lens proteins do not act the same as they do in vivo or even in vitro. Other studies (Wolbarsht, 1979) have indicated that there was a protective feature for α crystallin in the intact lens, even mixtures with other types of lens proteins appear to prevent infrared degradation of α crystallin. That is, α crystallin alone is degraded much more rapidly than in mixtures with other lens proteins.

CONCLUSIONS

The present work has indicated even more firmly that the IR cataractogenesis is a dose related phenomena (proportional to the total energy rather than the power level). The relation between power level and exposure duration holds until at least until 20 minutes. Although

this is a much shorter duration than the industrial or chronic exposures which have supposedly have led to infrared cataract, we hope to bridge the gap between the two exposure ranges with future experiments. In any case, the key step in cataractogenesis still seems to be the initial degradation of α crystallin as detected by thin layer gel electrophoresis.

POSSIBLE LINE OF FUTURE INVESTIGATION

Longer exposures and lower power levels will be used to determine if reciprocity holds over longer periods of time. With these longer exposure periods, fractionation of the dose, with exposure periods separated by days or even weeks, will also be used to determine the integration period. We planned to compare the data obtained from pulsed IR lasers such as gallium arsenide or other near-infrared lasers to the present results with CW IR lasers.

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